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Efficient generation of 2*E*-hexenal by a hydroperoxide lyase from mung bean seedlings

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2*E*-hexenal was generated with a high molar conversion rate by the incubation of a hydroperoxide lyase containing extract from mung bean seedlings and its substrate, 13-hydroperoxy-9*Z*,11*E*,15*Z*-octadecatrienoic acid (13-HPOT). Various parameters affected the yield of 2*E*-hexenal and the conversion rate: hydroperoxide lyase activity was especially, pronounced in seedlings older than 10 days. The cleavage of 13-HPOT by a solubilized enzyme extract proceeded best at pH 6.5. Time-course studies showed that the majority of flavour compounds was already generated during the first 10 min of the incubation period. Using optimized reaction conditions, a maximum yield of 1062 mg kg^{-1} 2*E*-hexenal was obtained with 32.6 mmol kg^{-1} (10.1 g kg^{-1}) 13-HPOT. However, the conversion rate was highest (maximum 86.3%) at a low substrate concentration, indicating a suicidal behaviour of the hydroperoxide lyase. Enzymatically hydrolyzed and dioxygenated linseed oil was a suitable alternative to pure linolenic acid. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

3*Z*- and 2*E*-hexenal are potent flavour compounds which contribute to the fresh 'green' odour of many vegetables and fruits. In plants, they are generated via the lipoxygenase pathway by a hydroperoxide lyase mediated cleavage of 13-hydroperoxy-9*Z*,11*E*,15*Z*-octadecatrienoic acid (13-HPOT) into 3*Z*-hexenal, which isomerizes enzymatically and/or chemically into the more stable 2*E*-isomer (Gardner, 1991; Hatanaka, 1996). Because of their volatility, they are easily lost during food processing. Hence, a reconstitution of the flavour of the manufactured food is required. Such a reconstitution can be achieved by the addition of chemically synthesized or natural hexenals. Aroma compounds are classified as 'natural' when they have been obtained from natural sources either by physical or fermentative processes. This includes the utilization of enzymes. As consumers in general prefer natural compounds, it is a challenging task for the food industry and biotechnology to provide them in sufficient amounts.

There exist several approaches in the literature to produce hexenals using either linolenic acid or 13-HPOT as the substrate. The formation of 3*Z*-/2*E*-hexenal by cell cultures of several fruits (Berger *et al.*, 1987) or alfalfa (Chou and Chin, 1994) has been described, but the yield was low (< 0.18 mg kg^{-1}). More effective was the utilization of quick grass (210 mg kg^{-1}) (Berger *et al.*, 1986), apple peel (571 mg kg^{-1}) (Drawert *et al.*, 1986), or the leaves of various plants (450 mg kg^{-1}) (Götz-Schmidt *et al.*, 1986). A screening of vegetables and fruits resulted in a maximum of hexenals of 370 mg kg^{-1} reaction mixture by green pepper (Whitehead *et al.*, 1995). Despite such high yields, the conversion rate was only moderate, especially when linolenic acid was applied as the substrate (5.9–16.1%).

The present work reports on the generation of 2*E*-hexenal using 13-HPOT, derived either from pure linolenic acid or from hydrolyzed and dioxygenated linseed oil as the substrate and mung bean seedlings as an enzyme source. Various parameters affecting the enzymatic reaction were investigated in order to improve not only the yield of 2*E*-hexenal, but also the efficiency of conversion.

MATERIALS AND METHODS

Mung beans (*Phaseolus radiatus* L.) and linseed oil were obtained from a local market. Linolenic acid (99%) was purchased from Fluka (Neu-Ulm, Germany). Lipoxygenase from soybean type 1-B (EC 1.13.11.12), lipase from *Candida cylindracea* type VII (EC 3.1.1.3), methyl *n*-heptanoate, methyl *n*-nonanoate,

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methyl *n*-heptadecanoate, and Triton X-100 were from Sigma (Deisenhofen, Germany).

Plant preparation and incubation

Mung bean seeds were germinated on moist cotton wool under normal light conditions for 10–14 days at room temperature. Generally, 5 g mung bean seedlings were homogenized in 50 ml 0.1 M potassium phosphate-citrate buffer pH 6.5 with an Ultraturrax (Janke and Kunkel, Staufen i. Br., Germany) for 90 s. The homogenate was incubated in sealed flasks with 50 μmol (10 mmol kg^{-1} seedlings) 13-HPOT for 1 h at room temperature in a circular agitator. In some experiments, the buffer contained 0.1% (w/v) Triton X-100 for the solubilization of the hydroperoxide lyase. This crude enzyme extract was used after centrifugation (6400*g*, 4°C, 20 min) for the incubation. For studies on the time-course of hexenal formation, the samples were treated at certain intervals with inhibitory amounts of methanol to a total content of 66% (v/v). Control samples (without the addition of 13-HPOT) were included in each experiment to consider 2*E*-hexenal formed by endogenous lipase and lipoxygenase from the plant material. All results are means of three experiments in which seedlings of the same origin, batch, and age were used. The relative standard deviation of all quantitative estimations of 2*E*-hexenal was approximately ± 5%.

Preparation of 13-HPOT

13-HPOT was prepared by enzymatic dioxygenation of linolenic acid using lipoxygenase-1 from soybean. This lipoxygenase generates almost exclusively the 13-hydroperoxide isomer at pH 9.0 and 0°C. Detailed reaction conditions and an analysis of 13-HPOT have been published elsewhere (Rehbock *et al.*, 1997). The results indicated a nearly complete conversion and isomeric purity > 95%.

Enzymatic hydrolysis and dioxygenation of linseed oil

One gram of linseed oil emulsified in 10 ml 0.1 M phosphate buffer pH 7.5 was hydrolyzed with the lipase of *Candida cylindracea* at 25°C for 2 h. Released free fatty acids were analysed after microextraction with *n*-hexane by titration with ethanolic KOH using phenolphthalein as an indicator. The total amount of fatty acids of the oil was determined after saponification with 0.5 M ethanolic KOH under reflux for 1 h. The degree of hydrolysis was calculated as the difference between total fatty acids and enzymatically released fatty acids divided by the total amount and multiplied by 100. The fatty acid composition of the oil was analysed after transesterification with Na-methylate by gas chromatography. Methyl *n*-heptadecanoate was used as a standard for the quantification of methyl linoleate and linolenate.

Dioxygenation of released linoleic and linolenic acid by the lipoxygenase of soybean was carried out as mentioned above. The resulting mixture, containing the 13-hydroperoxides of linolenic and linoleic acid, served as a substrate for an enzyme extract of mung bean seedlings in some experiments. The sum of hydroperoxides was determined spectrophotometrically at 234 nm using a molar extinction coefficient of 25 000 (Axelrod *et al.*, 1981). High performance liquid chromatography (HPLC) analysis of the hydroperoxides (Rehbock *et al.*, 1997) showed nearly the same ratio of 13-HPOT/13-HPOD (13-hydroperoxy-9*Z*,11*E*-octadecadienoic acid) as of the respective fatty acids. This indicated that soybean lipoxygenase dioxygenated both fatty acids to the same extent.

Isolation and analysis of flavour compounds

Methyl *n*-heptanoate was added to the reaction mixture as an internal standard in order to determine the recovery of products by solvent extraction. The reaction mixture was extracted three times with 70 ml pentane/diethyl ether (1:1, v/v). After drying over anhydrous sodium sulphate, the organic phases were concentrated to 1 ml by distillation using a Vigreux column (water bath temperature 40°C). Subsequently, methyl *n*-nonanoate was added as an external standard to the concentrate and used for calculating the recovery and the product concentration. A calibration curve was made for 2*E*-hexenal and methyl *n*-heptanoate using methyl *n*-nonanoate as a standard to consider the different detector response.

The metabolites were analysed on a Fisons gas chromatograph GC 8180 (Mainz, Germany) equipped with cool on-column injector, flame ionization detector (at 270°C), and Sato Wax capillary column (30 m × 0.32 mm, 0.45 μm film thickness) from Sato (Mönchengladbach, Germany). The temperature was programmed from 40°C (held for 2 min) to 220°C at 4°C min^{-1}. The carrier gas was hydrogen at an inlet pressure of 50 kPa.

RESULTS AND DISCUSSION

Age of seedlings

Mung bean seedlings were cultivated over a period of 17 days. At certain intervals they were examined for their ability to generate 2*E*-hexenal either from endogenous linolenic acid or exogenously applied 13-HPOT. With increasing age, the amount of 2*E*-hexenal derived from endogenous linolenic acid increased as well and reached a maximum of 49 mg kg^{-1} at day 12 (Fig. 1). A prolonged cultivation resulted in a slight decrease of 2*E*-hexenal formation. An increase of hydroperoxide lyase and lipoxygenase activity at an early stage of development, followed by declining activities, has also been reported for germinating soybeans (Olias *et al.*, 1990) and watermelon seedlings (Vick and Zimmerman,

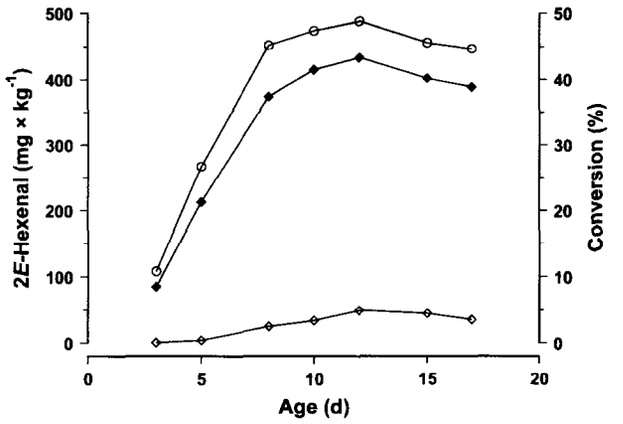

Fig. 1. Developmental changes in the generation of 2*E*-hexenal by homogenates of mung bean seedlings. Mung bean seedlings (5 g) at different stages of development were homogenized in 50 ml phosphate buffer (pH 6.5) and incubated with and without 8.0 mmol kg^{-1} 13-hydroperoxy-9*Z*,11*E*,15*Z*-octadecatrienoic acid (13-HPOT; derived from pure linolenic acid) at room temperature for 1 h. 2*E*-hexenal was determined after solvent extraction by gas chromatography. ◆, 2*E*-hexenal (+ 13-HPOT); ◇, 2*E*-hexenal (control); ○, molar conversion rate of 13-HPOT into 2*E*-hexenal.

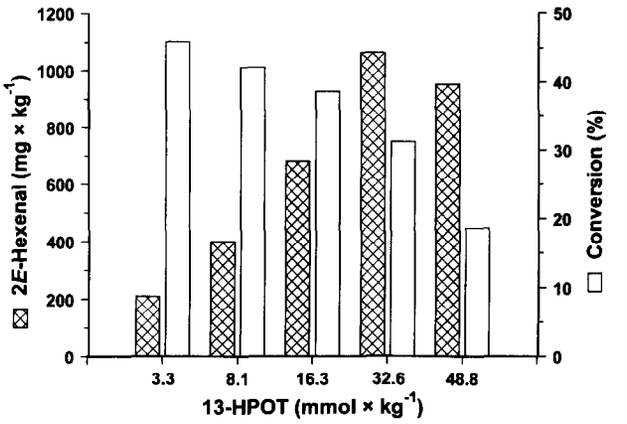

Fig. 2. Effect of precursor concentration on the formation of 2*E*-hexenal. Homogenates of 5 g mung bean seedlings were incubated at room temperature and at pH 6.5 with various amounts of 13-HPOT (derived from pure linolenic acid) for 1 h.

1976). After the addition of 13-HPOT, the age-dependent formation of 2*E*-hexenal was similar, but the yield was significantly improved (433 mg kg^{-1}). Compared with previously reported work (cf. Introduction), an enhanced conversion rate of 48.8% was obtained. This indicates that mung bean seedlings contain a particularly active hydroperoxide lyase.

Effect of 13-HPOT concentration

The effect of 13-HPOT on the yield of 2*E*-hexenal and on the efficiency of conversion is presented in Fig. 2. An enhanced amount of 13-HPOT resulted in an increased yield of 2*E*-hexenal with a maximum of 1062 mg kg^{-1}. The conversion rate showed an opposite trend, indicating probably an inhibition of the hydroperoxide lyase by its substrate or product. In an additional experiment, in which the buffer volume of the reaction mixture was enlarged from 50 to 200 ml, a yield of 402 mg kg^{-1} 2*E*-hexenal and a conversion rate of 86.3% was achieved using a substrate concentration of 4.28 mmol kg^{-1}. In order to establish a possible product inhibition, 2*E*-hexenal was added at a concentration of 560 mg kg^{-1} to a homogenate prior to the incubation with 13-HPOT, but no inhibitory effect could be detected. Therefore, a suicide-like inhibition of the lyase by the hydroperoxide seems to be more likely. Matsui *et al.* (1992) observed an irreversible inactivation of tea leaf hydroperoxide lyase by the 13- and 9-hydroperoxides of linoleic acid. Their proposed inactivation mechanism involved the conversion of the fatty acid hydroperoxide by the hydroperoxide lyase into a radical species which is thought to damage a sulfhydryl group essential for the enzyme activity. The presence of a sulfhydryl group in the active centre of various hydroperoxide lyases (Schreier and Lorenz, 1982; Matsui *et al.*, 1991; Shibata *et al.*, 1995) has been demonstrated by inhibition experiments using Hg^{2+}-ions or *p*-chloromercuryphenyl-sulphonic acid.

Solubilization of hydroperoxide lyase

With respect to a biotechnological process, the utilization of a particle-free enzyme extract instead of a homogenate appears to be more desirable. As hydroperoxide lyases in plants occur membrane-bound, a detergent is necessary for their solubilization. In order to obtain a particle-free lyase extract, the seedlings were homogenized in the presence of 0.1% (w/v) Triton X-100 and subsequently centrifuged. Within a range of 0.1–1.0% (w/v) tested, Triton X-100 at 0.5% (w/v) proved to be the most effective (data not shown). Nevertheless, Triton X-100 at a concentration of 0.1% (w/v) was used for the following reasons: firstly, the increase in hexenal formation at 0.5 (w/v) Triton X-100 compared with 0.1% (w/v) was 6% only. Secondly, the solvent extraction of the products proceeded more easily at a low detergent concentration.

Control samples consisted of an extract and a homogenate both prepared without detergent. After the addition of 11.1 mmol kg^{-1} 13-HPOT, the samples were incubated at pH 6.5 for 1 h. Triton X-100 was able to solubilize the hydroperoxide lyase of mung bean seedlings efficiently: the yield of 2*E*-hexenal generated by a Triton X-100 containing enzyme extract (543 mg kg^{-1}) exceeded slightly that of a homogenate lacking the detergent (533 mg kg^{-1}). In the extract, which was prepared without Triton X-100, the amount of 2*E*-hexenal was reduced to 187 mg kg^{-1}. Its formation in this sample was due to the presence of small membrane fragments bearing hydroperoxide lyase activity which were not removed by centrifugation at 6400*g*.

Effect of pH

The effect of pH on the generation of 2*E*-hexenal is shown in Fig. 3. Using 13-HPOT as the substrate, the cleavage into 2*E*-hexenal was best in the range from pH 6 to 7, with a slight optimum at pH 6.5 (522 mg kg^{-1}). On account of the insolubility of 13-HPOT at pH 4.0, the yield of 2*E*-hexenal was drastically reduced, being only one third compared with pH 6.5. The pH optimum of the hydroperoxide lyase of mung bean seedlings is in accordance with those previously reported for soybean seedlings (Olias *et al.*, 1990) and watermelon seedlings (Vick and Zimmerman, 1976), which were at pH 6–7 and 6–6.5, respectively. Hydroperoxide lyases from green bell pepper fruits (Shibata *et al.*, 1995) and tea leaves (Matsui *et al.*, 1991) possess a maximum of activity at pH 5.5 and 7.5, respectively.

Compared with experiments with the addition of a substrate, the generation of 2*E*-hexenal from endogenous linolenic acid exhibited an optimum at pH 4.5–5 (Fig. 3). As this process involves several enzymatic steps, namely a lipase-catalyzed release of linolenic acid from membrane lipids, dioxygenation of linolenic acid by a lipoxygenase, and subsequent cleavage of the fatty acid hydroperoxide by a hydroperoxide lyase, it was assumed that the optimum of the lipase or lipoxygenase from mung bean seedlings is in the more acidic range.

Time-course of hexenal formation

When a crude enzyme extract of the seedlings was incubated with 13-HPOT, the cleavage by the hydroperoxide lyase started rapidly (Fig. 4). Within the first 10 min, 72% of the final yield of hexenals was generated. An accumulation of the initial product 3*Z*-hexenal could not be observed: it isomerized enzymatically, catalyzed by an 3*Z*/2*E*-enal-isomerase or chemically into

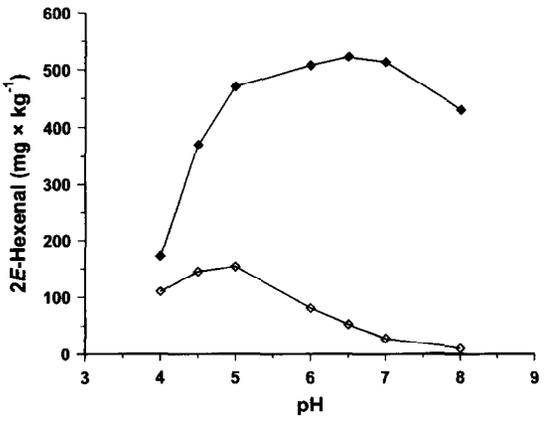

Fig. 3. pH optimum of 2*E*-hexenal formation. Crude enzyme extracts (0.1% w/v Triton X-100) of 5 g mung bean seedlings were incubated with and without 10.1 mmol kg^{-1} 13-hydroperoxy-9*Z*,11*E*,15*Z*-octadecatrienoic acid (13-HPOT; derived from pure linolenic acid) at various pH for 1 h at room temperature. ◆, 2*E*-hexenal (+ 13-HPOT); ◇, 2*E*-hexenal (control).

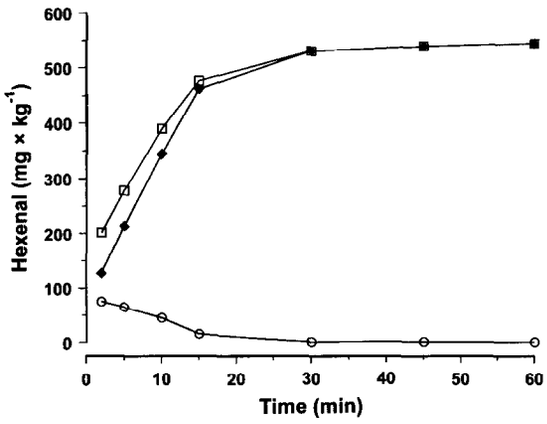

Fig. 4. Time-course of 3*Z*-/2*E*-hexenal formation. Crude enzyme extracts (0.1% w/v Triton X-100) of 5 g mung bean seedlings were incubated with 10.6 mmol kg^{-1} 13-hydroperoxy-9*Z*,11*E*,15*Z*-octadecatrienoic acid (13-HPOT; derived from pure linolenic acid) at room temperature and at pH 6.5 for various periods of time. ◆, 2*E*-hexenal; □, sum of 3*Z*- and 2*E*-hexenal; ○, 3*Z*-hexenal.

2*E*-hexenal. After a period of 30 min, the formation of hexenals stopped, although the molar conversion reached, at that point, 52% only.

An incomplete conversion of 13-HPOT into hexenals can be caused by inhibition of the hydroperoxide lyase or by generation of further metabolites. Additional volatiles, such as 3*Z*-hexenol or 2*E*-hexenol, which can derive from a reduction of hexenals by an alcohol dehydrogenase, were not detectable by gas chromatography. However, HPLC analysis with a particle beam-mass spectrometer and an evaporative light-scattering detector revealed the presence of several non-volatile oxylipins (Rehbock *et al.*, 1997). They were enzymatically formed during the metabolism of 13-HPOT catalyzed by an allene oxide synthase, allene oxide cyclase, and a peroxygenase. The contribution of these oxylipins to the hydroperoxide metabolism was approximately 15%. Further by-products may originate from the reaction of 2*E*-hexenal with plant proteins leading to Schiff bases (Gardner, 1979), from a formation of protein–hydroperoxide complexes (Hidalgo and Kinsella, 1989), or from polymerization of 13-HPOT (Gardner, 1987). These derivatives are not detectable with the analytical system used in this study.

Formation of 2*E*-hexenal and hexanal from linseed oil

One gram of linseed oil, a natural raw material with a high content of linolenic acid, was hydrolyzed by a non-specific lipase of *Candida cylindracea*. The oil used in this study contained 385 mg linolenic and 148 mg linoleic acid per gram of oil. The degree of enzymatic hydrolysis was approximately 90%, as determined by titration. After dioxygenation of released linolenic and linoleic acid by the lipoxygenase of soybeans, aliquots of the resulting hydroperoxide solution (yield 96%) were withdrawn and used as a substrate for an enzyme

Table 1. Generation of 2*E*-hexenal and hexanal from linseed oil

Linseed oil ($g\,kg^{-1}$)	13-HPOT ($mmol\,kg^{-1}$)	2*E*-hexenal ($mg\,kg^{-1}$)	Conversion of 13-HPOT (%)	13-HPOD ($mmol\,kg^{-1}$)	Hexanal ($mg\,kg^{-1}$)	Conversion of 13-HPOD (%)
0	0	105	0	0	5	0
10	12.0	561	38.7	4.6	103	21.3
20	24.0	778	28.6	9.2	57	5.6
30	36.0	797	19.6	13.9	35	2.2
40	48.0	770	14.1	18.5	30	1.4

13-HPOT = 13-hydroperoxy-9*Z*,11*E*,15*Z*-octadecatrienoic acid, 13-HPOD = 13-hydroperoxy-9*Z*,11*E*-octadecadienoic acid.

extract of 5 g mung bean seedlings. The control sample contained no linseed oil, but lipase and lipoxygenase which were also present in the substrate solution. The molar conversion rate was calculated as the difference between the molar amounts of aldehydes obtained in experiments with and without the addition of substrate divided by the molar substrate concentration multiplied by 100. All data are given in relation to 1 kg of seedlings. Table 1 presents the yields and conversion rates of 2*E*-hexenal and hexanal. After the addition of $20\,g\,kg^{-1}$ hydrolyzed and dioxygenated linseed oil, $797\,mg\,kg^{-1}$ 2*E*-hexenal were obtained. As the content of linoleic acid in the oil used in these experiments was 2.6-fold lower than linolenic acid, the maximum yield of hexanal was only $103\,mg\,kg^{-1}$. The conversion rates of both 13-HPOT and 13-HPOD decreased with increasing amounts of the oil, but not to the same extent. Obviously, the hydroperoxide lyase of mung bean seedlings prefers 13-HPOT as a substrate when both were offered simultaneously.

In summary, the enzymatic processes presented in this study allowed a rapid and efficient generation of 2*E*-hexenal. Contrary to prior investigation using free fatty acid as a substrate, the application of the hydroperoxide represented a significant improvement with respect to the conversion rates. Linolenic acid was successfully replaced by linseed oil, which proved to be a more suitable alternative from an economic point of view.

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REFERENCES

Axelrod, B., Cheesbrough, T. M. and Laakso, S. (1981) Lipoxygenase from soybeans. *Methods in Enzymology* **71**, 441–451.

Berger, R. G., Kler, A. and Drawert, F. (1986) The C_6-aldehyde-forming system in *Agropyron repens*. *Biochimica et Biophysica Acta* **883**, 523–530.

Berger, R. G., Kler, A. and Drawert, F. (1987) C_6-aldehyde formation from linolenic acid in fruit cells cultured in vitro. *Plant Cell, Tissue and Organ Culture* **8**, 147–151.

Chou, S.-R. and Chin, C.-K. (1994) Control of the production of cis-3-hexenal, lipid-derived flavor compound, by plant cell culture. In *Lipids in Food Flavors*, eds C-T. Ho and T. G. Hartmann, pp. 282–291, ACS, Washington, DC.

Drawert, F., Kler, A. and Berger, R. G. (1986) Biotechnologische Erzeugung von Aromastoffen. I. Optimierung der Ausbeuten von (E)-2-Hexenal bei pflanzlichen Gewebehomogenaten. *Lebensmittel-Wissenschaft und-Technologie* **19**, 426–431.

Gardner, H. W. (1991) Recent investigations into the lipoxygenase pathway of plants. *Biochimica et Biophysica Acta* **1084**, 221–239.

Gardner, H. W. (1987) Reactions of hydroperoxides—products of high molecular weight. In *Autoxidation of Unsaturated Lipids*, ed. H. W.-S. Chan, pp. 51–94. Academic Press, London.

Gardner, H. W. (1979) Lipid hydroperoxide reactivity with proteins and amino acids: a review. *Journal of Agricultural and Food Chemistry* **27**, 220–229.

Götz-Schmidt, E.-M., Wenzel, M. and Schreier, P. (1986) C_6-volatiles in homogenates from green leaves: localization of hydroperoxide lyase activity. *Lebensmittel-Wissenschaft und-Technologie* **19**, 152–155.

Hatanaka, A. (1996) The fresh green odor emitted by plants. *Food Reviews International* **12**, 303–350.

Hidalgo, F. J. and Kinsella, J. E. (1989) Changes induced in β-lactoglobulin B following interactions with linoleic acid 13-hydroperoxide. *Journal of Agricultural and Food Chemistry* **37**, 860–866.

Matsui, K., Kajiwara, T. and Hatanaka, A. (1992) Inactivation of tea leaf hydroperoxide lyase by fatty acid hydroperoxide. *Journal of Agricultural and Food Chemistry* **40**, 175–178.

Matsui, K., Toyota, H., Kajiwara, T., Kakuno, T. and Hatanaka, A. (1991) Fatty acid hydroperoxide cleaving enzyme, hydroperoxide lyase, from tea leaves. *Phytochemistry* **30**, 2109–2112.

Olias, J. M., Rios, J. J., Valle, M., Zamora, R., Sanz, L. C. and Axelrod, B. (1990) Fatty acid hydroperoxide lyase in germinating soybean seedlings. *Journal of Agricultural and Food Chemistry* **38**, 624–629.

Rehbock, B., Ganßer, D. and Berger, R. G. (1997) Analysis of oxylipins by high performance liquid chromatography with evaporative light-scattering detection and particle beam-mass spectrometry. *Lipids* **32**, 1003–1010.

Shibata, Y., Matsui, K., Kajiwara, T. and Hatanaka, A. (1995) Purification of fatty acid hydroperoxide lyase from green bell pepper fruits. *Plant Cell Physiology* **36**, 147–156.

Schreier, P. and Lorenz, G. (1982) Separation, partial purification and characterization of a fatty acid hydroperoxide cleaving enzyme from apple and tomato fruits. *Zeitschrift für Naturforschung* **37c**, 165–173.

Vick, B. A. and Zimmerman, D. C. (1976) Lipoxygenase and hydroperoxide lyase in germinating watermelon seedlings. *Plant Physiology* **57**, 780–788.

Whitehead, I. M., Muller, B. L. and Dean, C. (1995) Industrial use of soybean lipoxygenase for the production of natural green note flavor compounds. *Cereal Foods World* **40**, 193–197.